Science of the Total Environment 653 (2019) 995–1004

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

N_2 fixation associated with the bryophyte layer is suppressed by low levels of nitrogen deposition in boreal forests

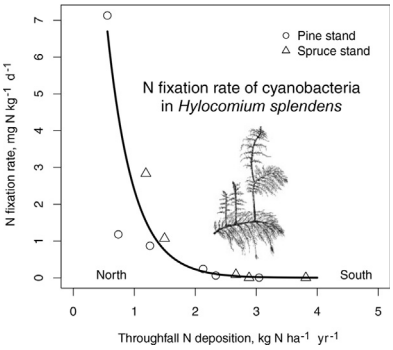
Maija Salemaa [a,*], Antti-Jussi Lindroos [a], Päivi Merilä [b], Raisa Mäkipää [a], Aino Smolander [a]

[a] Natural Resources Institute Finland (Luke), Latokartanonkaari 9, FI-00790 Helsinki, Finland
[b] Natural Resources Institute Finland (Luke), P.O. Box 413, FI-90570 Oulu, Finland

HIGHLIGHTS

- Biological nitrogen fixation (BNF) associated with mosses and lichens was studied.
- Tree canopies controlled the N deposition affecting BNF rate.
- Mainly inorganic N, but also dissolved organic N had negative effect on BNF.
- N deposition threshold suppressing BNF gives data to setup of critical N load.
- Climate change affects BNF rates.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 27 August 2018
Received in revised form 25 October 2018
Accepted 27 October 2018
Available online 29 October 2018

Editor: Elena PAOLETTI

Keywords:
Canopy effect
Climate change
Critical load
Cyanobacteria
Bio-indicator
N fixation capacity

ABSTRACT

Biological fixation of atmospheric nitrogen (N_2) by bryophyte-associated cyanobacteria is an important source of plant-available N in the boreal biome. Information on the factors that drive biological N_2 fixation (BNF) rates is needed in order to understand the N dynamics of forests under a changing climate. We assessed the potential of several cryptogam species (the feather mosses *Hylocomium splendens* and *Pleurozium schreberi*, a group of *Dicranum* bryophytes, two liverworts, and *Cladina* lichens) to serve as associates of cyanobacteria or other N_2-fixing bacteria (diazotrophs) using acetylene reduction assay (ARA). We tested the hypotheses that the legacy of chronic atmospheric N deposition reduces BNF in the three bryophyte species, sampled from 12 coniferous forests located at latitudes 60–68° N in Finland. In addition, we tested the effect of moisture and temperature on BNF. All species studied showed a BNF signal in the north, with the highest rates in feather mosses. In moss samples taken along the north–south gradient with an increasing N bulk deposition from 0.8 to 4.4 kg ha^{-1} $year^{-1}$, we found a clear decrease in BNF in both feather mosses and *Dicranum* group. BNF turned off at N deposition of 3–4 kg ha^{-1} $year^{-1}$. Inorganic N (NH_4-N + NO_3-N) best predicted the BNF rate among regression models with different forms of N deposition as explanatory variables. However, in southern spruce stands, tree canopies modified the N in throughfall so that dissolved organic N (DON) leached from canopies compensated for inorganic N retained therein. Here, both DON and inorganic N negatively affected BNF in *H. splendens*. In laboratory experiments, BNF increased with increasing temperature and moisture. Our results suggest that even relatively low N deposition suppresses BNF in bryophyte-associated diazotrophs. Further, BNF could increase in northern low-deposition areas, especially if climate warming leads to moister conditions, as predicted.

* Corresponding author.
E-mail address: maija.salemaa@luke.fi (M. Salemaa).

https://doi.org/10.1016/j.scitotenv.2018.10.364
0048-9697/

1. Introduction

In northern forests with limited nitrogen (N), biological fixation of N_2 gas (BNF) by bryophyte- and lichen-associated cyanobacteria is an important source of plant-available N, adding up to 1–2 kg N ha^{-1} $year^{-1}$ to the ecosystem (DeLuca et al., 2002; Zackrisson et al., 2004; Leppänen et al., 2013; Rikkinen, 2017). The role of bryophyte-cyanobacteria associations is essential in processes that determine whether the northernmost ecosystems become C sinks or C sources in response to global change, because they can control both net primary productivity and heterotrophic respiration (DeLuca et al., 2008; Gundale et al., 2011, 2013; Lindo et al., 2013; Rousk et al., 2013a). Knowledge of the forces driving BNF rates of bryophyte-cyanobacteria is crucial when modelling the response of the N and C dynamics of northern forests to a changing climate.

BNF by cyanobacteria and other N_2-fixing bacteria is an energy-expensive process mediated by the activity of the enzyme nitrogenase. Several studies carried out in boreal forests have shown that BNF rate is restricted by the ambient N level: A declining trend of BNF with increasing atmospheric N deposition has been found in large-scale surveys (DeLuca et al., 2002) and in areas affected by traffic (Ackermann et al., 2012). Similarly, increased N availability after forest cuttings (Stuiver et al., 2015), after experimental N additions in forests (Zackrisson et al., 2004; Gundale et al., 2011, 2013), and in laboratory experiments (Rousk et al., 2014a) have expressed a negative relationship between BNF rate and external N supply. However, in a long-term N fertilization study, high amounts of ammonium (NH_4) and nitrate (NO_3) did not suppress BNF in moss species in a bog in Scotland (van den Elzen et al., 2018). Further, cyanobacterial communities have shown to recover from very high N additions during the short periods of N deprivation after exposure (Rousk and Michelsen, 2016).

The studies on the effects of added N on cyanobacterial activity carried out as field or laboratory experiments have applied variable amounts and forms of given N, which makes it difficult to directly compare their results to each other (Rousk et al., 2013a). In explorative large-scale surveys, information on N deposition is often based on few measuring points and approximated over large areas (e.g., DeLuca et al., 2002). In fertilization experiments, it is sometimes difficult to assess the actual amount of N to which bryophytes are exposed because precipitation may wash and dilute the given solution, or fertilization grains may become spread unevenly on the ground. The BNF rate also depends on the cyanobacterial communities, which show a high degree of host specificity (Ininbergs et al., 2011) and may vary depending on the long-term deposition history (Rousk et al., 2014a) or succession stage of the home forest (Zackrisson et al., 2004). As a consequence, the estimates suggested for the threshold amount of N being able to suppress cyanobacterial activity vary between different studies, ranging from 3 to over 10 kg N ha^{-1} $year^{-1}$ (reviewed in Rousk et al., 2013a). Because most experimental additions of N are higher than the prevailing atmospheric N load in the northern boreal zone, there is a need to study the BNF capacity of cryptogam species collected from field sites under actual deposition levels. This information is needed in calculating boreal forest N budgets and in predicting the effects of climate change on N dynamics in boreal forests.

Atmospheric N deposition in Finland is considerably lower than in Central Europe where it can reach over 10 kg ha^{-1} $year^{-1}$ in many locations (Vuorenmaa et al., 2018). The lowest N deposition levels in Finland are found in the northern part of the country (<1.5 kg ha^{-1} $year^{-1}$), whereas anthropogenic N emissions from road traffic, energy production and agriculture have resulted in elevated N deposition levels in the south (Mustajärvi et al., 2008; Waldner et al., 2014; Vuorenmaa et al., 2018). Lately, N deposition loads have shown a decreasing trend in Europe (Waldner et al., 2014; Vuorenmaa et al., 2017); in Finland, however, the change has been minor (Mustajärvi et al., 2008, Waldner et al., 2014, Vuorenmaa et al., 2018). In forests characterized by limited N availability, as in Finland, a common phenomenon is that tree canopies retain inorganic forms of N (NH_4-N, NO_3-N) from deposition, but the flux of dissolved organic nitrogen (DON) correspondingly increases as rainwater passes through the canopy layer as stand throughfall (TF). This is due to dry deposited DON being washed off, or transformation processes between inorganic N and DON (Mustajärvi et al., 2008; Ferm and Hultberg, 1999). The pool of DON in TF is a mixture of simple, N-containing amino acids and complex, organic compounds released from tree foliage and epiphytes (Cornell et al., 2003). It also contains terrestrial dust particles and plant pollen caught by the canopy (Neff et al., 2002). Depending on the site and stand characteristics, the quantity and composition of forms of N between bulk deposition and TF entering the bryophyte layer thus differ from each other. It is well known that inorganic N has a negative effect on BNF in boreal bryophytes (e.g. Zackrisson et al., 2004; Gundale et al., 2013), but only a few studies have focused on the role of organic N as a controlling factor (DeLuca et al., 2008; Rousk et al., 2013b).

The BNF rate is also influenced by moisture (Gundale et al., 2012a; Rousk et al., 2014b), temperature and light (Gundale et al., 2012b), and CO_2 concentrations (Lindo and Griffith, 2017). Currently, the effects of temperature and moisture are of special interest, because global climate change models predict considerable warming and increased precipitation, but also more severe drought periods, especially at northern latitudes (IPCC, 2013; Ruosteenoja et al., 2016). Furthermore, when calculating the annual N input estimates at stand level, information on the period of favorable field conditions for BNF activity in diazotrophs is required. As poikilohydric plants, forest bryophytes cannot regulate their water content, and they dry out during periods of low precipitation (Proctor et al., 2007), which may limit BNF activity.

In this study, we determined the potential of different cryptogam species – including mosses, liverworts and reindeer lichens – to host cyanobacteria or other N_2-fixing diazotrophs in an area of low N deposition level in the boreal zone. The feather mosses *Hylocomium splendens* (Hedw.) Schimp. and *Pleurozium schreberi* (Brid.) Mitt., as well as a group of acrocarpic *Dicranum* species were sampled from intensively monitored forest plots along a climatic gradient in Finland. We studied how their BNF rate was related to the legacy of N deposition that the mosses have been exposed to in their original growing sites, when the samples were incubated in a common environment. Our aim was to determine the actual N deposition level prevailing in coniferous forests that turns off BNF in diazotrophs associated with bryophytes. We also studied the effect of moisture and temperature on BNF rates in smaller data sets in the laboratory. We tested the following three hypotheses:

(1) The BNF rate of bryophyte-diazotroph associations decreases along a north–south gradient with increasing atmospheric N deposition.
(2) The total amount of N (NH_4-N + NO_3-N + DON), in both bulk and TF precipitation, predicts the variation in BNF rate better than inorganic N (NH_4-N + NO_3-N) alone.
(3) Increased temperature and moisture enhance the BNF rate.

The first and third hypotheses are based on earlier studies testing the effects of nitrogen (DeLuca et al., 2002; Zackrisson et al., 2004; Ackermann et al., 2012, Gundale et al., 2013), temperature (Gundale et al., 2012a, 2012b), and moisture (Smith and Russell, 1982; Gundale et al., 2009) on BNF in feather mosses. Our study gives new information by relating the BNF rates of bryophytes to the N deposition that the bryophytes and their associated diazotrophic bacteria have been chronically exposed to in the field. Quantitative data on different forms of N and long-term monitoring of N deposition in boreal forests enabled us to evaluate the importance of both inorganic N and DON as controlling factors for BNF rates.

2. Material and methods

2.1. Study sites

We collected bryophyte and lichen samples from 12 intensively monitored coniferous forest plots (UN-ECE ICP Forests Level II network,

Merilä et al., 2014) along a latitudinal gradient in the boreal zone in Finland (Fig. 1, Tables A.1–A.4). The total plot area at each site was 0.5 ha, consisting of 2–3 subplots, each sized 30 × 30 m. Seven of the plots were dominated by Scots pine (*Pinus sylvestris* L.) and five by Norway spruce (*Picea abies* L. H. Karst.). In five locations, pine and spruce plots were situated near each other. Basic information about the stand and site characteristics is given in Table 1. The annual mean temperature (years 2006–2010) of the study sites derived from model interpolations from the Finnish Meteorological Institute (Venäläinen et al., 2005) ranged from −1.1 °C to 5.2 °C. The age of the stands varied from 60 to 80 years, except the most northern ones, which were over 100 years old. The northernmost plot (no. 1) was located in a protected area, and the others were under conventional forestry practices. In three plots, stand thinning had been carried out not long before our sampling (Table 1). Understory vegetation consisted of typical boreal dwarf shrubs, herbs, grasses, bryophytes and lichens (Salemaa et al., 2008).

2.2. Precipitation and N deposition measurements

Monitoring data on the amount of precipitation and chemical composition of deposition on the open areas (bulk deposition) was available for 11 plots (Mustajärvi et al., 2008). For pine and spruce plots that were located close to each other, there was one common plot for open area deposition measurements. For plot no. 1, we used NH_4-N and NO_3-N deposition data measured from the same research area in 1995 as part of the UN/ECE Integrated Monitoring Programme (Ruoho-Airola et al., 1998). In our study, dissolved organic N (DON) deposition was modelled according to the inorganic N/DON ratio of the other pine plots in northern Finland. Stand TF deposition was available for ten plots. For pine plots nos. 1 and 3, we modelled the amount and forms of N in TF deposition according to the ratios in plot no. 5. Because of annual variation in the amounts of precipitation and N deposition, we used mean deposition values from the period 2006–2010 (for plot no. 1, data from 1995).

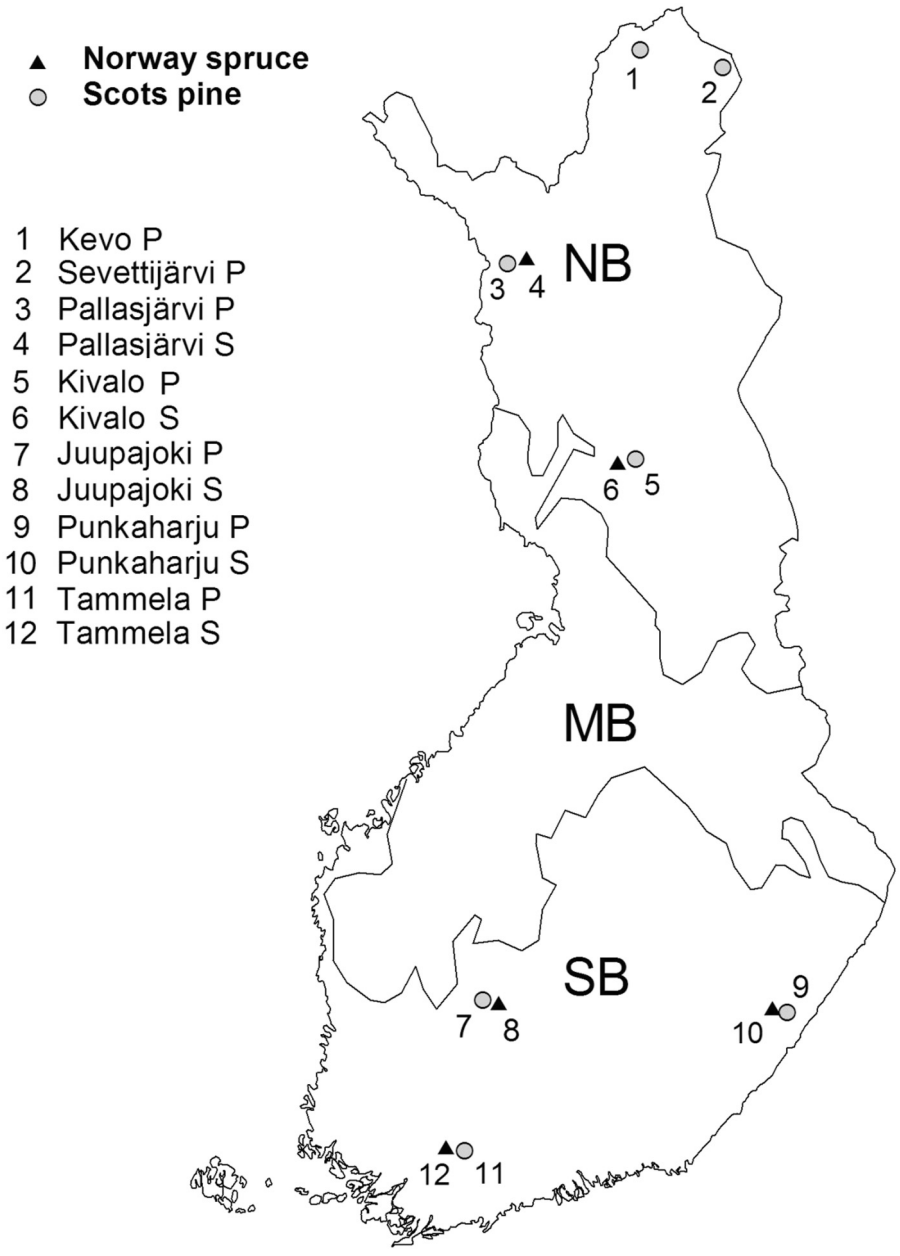


Fig. 1. Sample plot locations in Finland. NB = northern boreal, MB = middle boreal and SB = southern boreal climatic subzones. S = Norway spruce and P = Scots pine in the names of study sites.

Table 1
Site and stand characteristics of the study plots. Mean temperature (T) per year and in July, and mean precipitation sum per year were calculated over 2006–2010. Stand and understory vegetation data measured in the year 2009. % = visually estimated cover %.

Plot no.	ICP plot no.	N latitude	Tree species	Mean T year	Mean T July	Mean precipitation	Stand age	Stand basal area	Tree canopy	Bryophytes	Lichens	Dwarf shrubs	Herbs & grasses
				°C	°C	mm $year^{-1}$	year	m^2 ha^{-1}	%	%	%	%	%
1	Kevo22	69° 44′	Pine	−1.1	11.7	518.7	185	5.11	6	44	10	52	0.4
2	Sevettijärvi1	69° 34′	Pine	−0.1	12.0	404.3	200	13.52	35	17	50	41	0
3	Pallasjärvi2	67° 57′	Pine	−0.4	13.4	539.2	100	17.85	21	74	10	39	0.2
4	Pallasjärvi3	67° 59′	Spruce	−0.4	13.1	539.2	140	15.42	40	93	0.2	53	1.5
5	Kivalo6[b]	66° 19′	Pine	1.1	14.9	565.2	55	18.94	23	84	0.5	22	0.1
6	Kivalo5[a]	66° 21′	Spruce	1.1	14.9	536.0	70	17.95	33	90	0.1	46	0.5
7	Juupajoki10	61° 52′	Pine	4.1	16.5	566.8	80	23.55	29	90	0.1	60	4.2
8	Juupajoki11[a]	61° 51′	Spruce	4.1	16.5	566.8	80	30.40	35	57	0.1	25	59.4
9	Punkaharju16[a]	61° 46′	Pine	4.2	17.8	645.0	80	31.95	60	96	0	28	1.9
10	Punkaharju17	61° 48′	Spruce	4.2	17.8	645.0	70	30.76	42	75	0	4	16.8
11	Tammela13	60° 36′	Pine	5.3	17.7	661.0	60	29.27	34	60	0.2	34	11.0
12	Tammela12	60° 38′	Spruce	5.2	17.6	680.8	60	33.08	69	72	0.2	53	7.1

[a] Stand thinning in 2005–2006.
[b] Stand thinning 2008.

The sampling procedure for the deposition measurements, including laboratory analyses, is described in detail in Mustajärvi et al. (2008). TF was collected within the forest stand with 20 rainfall collectors, and BD in the nearby open area with 3 rainfall collectors (funnel, diameter 20 cm), during the snow-free period. During winter, 6–10 (TF) and 2 (BD) snow collectors (diameter 36 cm) were used. Samples were collected at 2–4 week intervals. Deposition samples were filtered (membrane filter, 0.45 μm). Total N was determined by flow injection analysis (FIA), and NH_4-N and NO_3-N by ion chromatography (IC). DON deposition was calculated by subtracting the measured NH_4-N and NO_3-N from the total N. The five-year means for total N in bulk and TF deposition are given in Table 2, and for different forms of N in Fig. 2. The annual data (years 2006–2010 separately) for the amounts of precipitation and different forms of N (total N, NH_4-N, NO_3-N and DON) in bulk and TF deposition are given in Table B.

2.3. Bryophyte and lichen sampling in field

Bryophyte and lichen samples for BNF analysis were collected in Aug–Sep in the years 2009, 2010 and 2013. The total number of sample plots was 12. Feather mosses were sampled from four plots both in 2009 and 2010, from two plots only in 2009, and from six plots only in 2010. *Dicranum* species were collected from three southern pine plots (2010) and from all six northern plots (2013). Samples from the liverwort species *Barbilophozia lycopodioides* were taken from two northern plots (nos. 1 and 2, 2009). Samples from another liverwort species, *Ptilidium ciliare*, were taken from a southern plot (2009) and a northern plot (2010). Lichens (*Cladina* group, mainly *Cladina stellaris*) were collected from two northern pine plots (nos. 2 and 3, 2013). Details of the species sampled in different years, species name abbreviations, and scientific names with authors are given in Tables A.1–A.4.

The bryophyte and lichen samples were collected as single-species samples in 500 ml containers. In 2009 and 2013, the number of bryophyte sampling points per plot was five (the distance between subjectively selected points was at least 5 m), and in 2010, samples were collected from 15 points per plot using systematic sampling (points at 7 m distances from each other along transects on four sides of a subplot with TF collectors). The samples from different locations were transported to Luke's laboratory in southern Finland and stored in a dark room at 5 °C (for 1–2 weeks) before incubation in similar external conditions per treatment. The incubated sample consisted of a fixed number of shoots (30 shoots of *P. schreberi*, 15 shoots of *H. splendens*, and 30–40 shoots of *Dicranum* spp. and of the other bryophyte species (discrete podetia in lichens)), which were placed into incubation bottles (125 ml glass bottles) and weighed in fresh. The incubated samples represented spatially separate field samples in 2009 and 2013. In 2010, we prepared three pooled samples by including shoots from all 15 field points in one incubation sample (using the method described in Leppänen et al., 2013). Thus, the number of incubated samples (hereafter called "samples") per plot and per species was five in the years 2009 and 2013, and three in 2010. For *H. splendens* and *P. schreberi*, only the upper green part of the shoots (length 4 cm, with 3–4 annual growths)

Table 2
Characteristics of the organic soil layer (year 2013), quantity of total N (NH_4-N + NO_3-N + DON) in the bulk and throughfall (TF) deposition (mean in 2006–2010), and the mean N_2 fixation rates of diazotrophic bacteria growing on different cryptogam host species (outdoor incubation in the same place, details of the N_2 fixation data in Table A.1).

Plot no.	Organic soil layer			N deposition, kg ha^{-1} $year^{-1}$		N_2 fixing of diazotrophs, mg N kg^{-1} d^{-1}				
	C/N ratio	N %	pH (H_2O)	Ntot bulk	Ntot TF	*Dicranum* spp.	*Hylo sple*	*Pleu schr*	*Barb lyco*	*Cladina* spp.
1	39.7	1.13	3.9	0.858	0.599	0.03	7.13	2.70	1.07	
2	50.2	0.77	3.8	1.363	0.740	0.02	1.18	4.16	1.26	0.01
3	59.4	0.59	3.9	1.294	0.844	0.02		0.58		0.01
4	46.3	1.00	4.0	1.294	1.190	0.02	2.83	2.31		
5	46.8	0.74	3.7	1.926	1.256	0.00	0.87	0.18		
6	41.3	0.96	3.9	1.788	1.496	0.00	1.07	0.00		
7	35.4	1.05	3.8	2.996	2.338	0.00	0.07	0.00		
8	34.9	0.87	4.2	2.996	2.666		0.09	0.00		
9	35.1	0.89	4.0	3.518	2.130	0.00	0.24	0.00		
10	31.7	1.30	3.8	3.518	2.882		0.00	0.00		
11	34.0	0.96	4.2	4.332	3.046	0.00	0.02	0.00		
12	30.5	1.27	3.9	4.376	3.812		0.00	0.00		

Dicranum spp. = *Dicranum* group bryophytes (Table A.4), *Hylo sple* = *Hylocomium splendens*, *Pleu schr* = *Pleurozium schreberi*, *Barb lyco* = *Barbilophozia lycopodioides* and *Cladina* spp. = reindeer lichens (mostly *Cladina stellaris*).

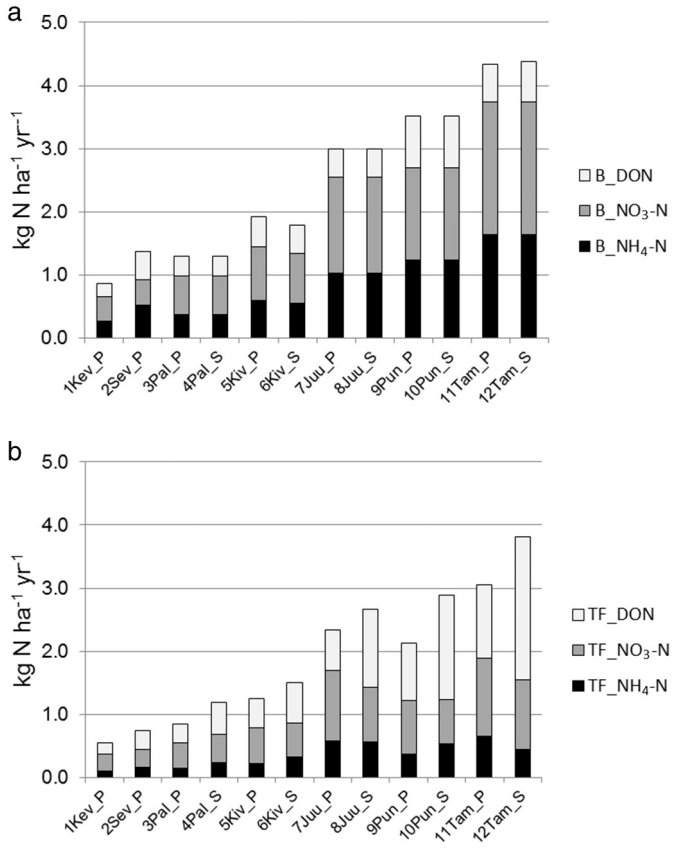


Fig. 2. Nitrogen forms (NH_4-N, NO_3-N and DON) in a) bulk (B) and b) throughfall (TF) deposition (mean across the years 2006–2010, kg ha^{-1} $year^{-1}$). P = Scots pine and S = Norway spruce in the names of study sites. Sites ordered along the climatic gradient from north (left) to south (right).

was used. In addition, in two spruce plots (nos. 4 and 12), we also incubated samples taken from the basal, 3–6 cm long, yellowish part of the shoots that was left after cutting the upper part (the ratio between the BNF rate of upper and lower parts was used in modelling the BNF rate for lower parts; see Section 2.5). For the *Dicranum*, liverwort and *Cladina* groups, we used the whole living thallus. *Dicranum* samples represented *D. polysetum* in the south and several *Dicranum* species in the north. (There is a complete list of species in Table A.4.)

2.4. Incubations and ARA measurements in different treatments

BNF rates of diazotrophs associated with bryophyte (and lichen) species were measured in three treatments – called (1) "site effect," (2) "temperature effect," and (3) "moisture effect" – using acetylene reduction assay (ARA). In this assay, the addition of acetylene (C_2H_2) to incubation bottles makes the nitrogenase enzyme of N_2-fixing bacteria reduce acetylene to ethylene (C_2H_4), instead of reducing N_2 to NH_4 (details of the method used are explained in Leppänen et al., 2013). ARA measures the activity of nitrogenase, correlating to N_2 fixation. No endogenous ethylene production was detected. At the end of the incubation, the dry mass of the samples was determined. We used the ^{15}N calibrating equation, based partly on the same samples with the current study (feather mosses from a northern spruce plot, no. 4) (Leppänen et al., 2013). Here, 3.3 mol of reduced acetylene corresponded to 1 mol of fixed N_2.

It is known that the incubation time affects the growth cycle of cyanobacteria populations (Lindo and Griffith, 2017). We repeated ARA measurements two or three times for the same samples, giving them two days "rest" without acetylene between the incubations. The mean of the two last measurements was used in the further analyses. Before incubation, all samples used in the three treatments were pretreated in a similar way: distilled water (2 ml) was added to the incubation bottles to standardize the moisture level, and the samples were kept at room temperature (20 °C) in natural light for 24 h.

2.4.1. Treatment 1 – "site effect": incubation in common outdoor conditions

We assumed that diazotroph activity was strongly determined by the legacy of plot-specific N deposition that the diazotrophs had been exposed to in the field before sampling, as has been shown in several earlier studies (see Section 1). In order to minimize the effects of other external factors (e.g. light and temperature) than the N deposition of the original growing site, we acclimatized and incubated all the samples in similar outdoor conditions in the institute yard in Vantaa, Finland.

The samples were transferred outdoors for 48 h to acclimatize to natural light and temperature of half-shade conditions in a common (treeless) yard. During the 24 h incubation with acetylene, the mean temperature was 9.5 °C (min. 4.5 °C, max. 13.5 °C) and the photosynthetically active radiation was 120–160 μmol s^{-1} m^{-2} (August 2010). The *Dicranum*, liverwort and *Cladina* samples were acclimatized and incubated in outdoor conditions at lower temperatures (mean 6 °C, min. 2 °C, max. 10 °C) in early October 2013. Outdoor temperatures in the yard area were relatively low in the late summer, and probably quite near the temperatures prevailing inside the moss layer in field conditions. We used the results from outdoor-incubated samples in comparing BNF capacities between the species and in calculating the response functions for three bryophyte species between BNF rate and different forms of N deposition measured in the original forests that the samples came from. The outdoor results were also used as a low-temperature reference to the BNF rates of samples incubated at higher temperatures (see below).

2.4.2. Treatment 2 – "temperature effect": incubation in growth chamber or indoor conditions

A set of samples of *H. splendens* and *P. schreberi* (and some other bryophyte species) was collected in late September 2009 from the northern plots (the species and plots are given in detail in Table A.2). The samples were acclimatized for one week in growth chamber (Conviron) conditions with temperatures of 23 °C in the day and 18 °C at night, and 19 h of light (radiation 280 μmol s^{-1} m^{-2}) and 5 h of dark before incubation with acetylene (24 h) and ARA measurements. The effect of temperature on BNF rates was studied by comparing these "high temperature" samples to those incubated outdoors (mean 9.5 °C, "low temperature"). For *Dicranum* spp. (2013), BNF rates were measured at two temperatures from the same samples, first outdoors (6 °C "low temperature") and then indoors (20 °C "high temperature") in laboratory conditions under natural light. Because *Dicranum* spp. had very low BNF rates, we used a longer (48 h) incubation time for it than for the other species (24 h).

2.4.3. Treatment 3 – "moisture effect": incubation outdoors with added water

The effect of moisture on the BNF rate of bryophyte-cyanobacteria was studied using *P. schreberi* shoots from the northern spruce plot no. 4 (2010). We treated the shoots in incubation bottles using five moisture levels (air-dried, field moisture, and addition of 1 ml, 2 ml or 5 ml of distilled water) with four replicate samples per treatment. Samples were otherwise acclimatized and measured as described above for outdoor incubations.

2.5. BNF rate calculations

BNF rates were calculated per dry weight of bryophyte mass (kg) and per day (d, 24 h). The detection limit for ARA measurements was 0.01 mg N $kg^{-1}d^{-1}$. The ratios between BNF of upper part to lower part (2.2 for *H. splendens* and 4.3 for *P. schreberi* according to the samples

from plot no. 4) were used in modelling BNF rates for lower parts. The BNF sum of the upper and lower parts was used in the further analyses. For *Dicranum* spp., we used the whole thallus in ARA incubations, but in test material (data not shown), the lower parts showed higher BNF rates than the upper parts.

3. Data analysis

We calculated plot-means of BNF rates (mg N kg^{-1} d^{-1}) from the replicate samples for each species (Tables A.1–A.3). Standard errors of mean (SE) were given only for the upper parts of the shoots, because the lower parts were modelled according to them. When samples were taken in two years, we calculated plot-means (±SE) across the years.

We used (a) one-factor ANOVA in comparing BNF rates of replicate samples (data from all years) of coexisting bryophyte, liverwort and lichen species in joint data of two northern plots, and (b) three-factor ANOVA in testing the effect of the host species (*H. splendens*, *P. schreberi*), latitude (north, south), and dominant tree species (pine, spruce) and their interactions on the BNF plot-means (11 plots with two coexisting species, in 2009 and 2010; *Dicranum* spp. was not included because of incomplete data). In addition, we tested (c) the effect of temperature during the acclimatizing and incubation period in feather mosses (outdoor "low temperature" vs. chamber "high temperature") and in *Dicranum* spp. (outdoor "low temperature" vs. indoor "high temperature") on the selected northern plots using replicate samples, and (d) the effect of moisture (five treatment levels with four replications) on the BNF rate of *P. schreberi* (data from plot no. 4) by one-factor ANOVA. Pairwise comparisons of BNF rates between the (a) species, (c) temperatures, and (d) moisture levels after ANOVA were made by post-hoc Tukey's *t*-tests or contrast analyses. BNF rates were log(x + 1) transformed in the analyses. All tests were carried out using the STATISTIX 10.0 program (2013).

We fitted generalized linear models (GLM, with a log link and the error distribution of Gamma family) for the three bryophyte species in order to answer whether the plot-mean of BNF rate (response variable, y) of bryophyte-diazotroph association decreases with increasing N deposition level (predictor, x) from north to south (the first hypothesis). We fitted equations having different forms of N (N_{tot} = NH_4-N + NO_3-N + DON, N_{inorg} = NH_4-N + NO_3-N or individually NH_4-N, NO_3-N and DON) as predictors, using bulk and TF deposition data in separate models. Zero values for BNF rate were substituted with a value of 0.005 (i.e. half of the detection limit of 0.01 mg N kg^{-1} d^{-1} for ARA). We tested the second hypothesis by comparing two GLMs, the first having only N_{inorg} as a predictor, and the second having both N_{inorg} and DON as predictors of the BNF rate (separate models were made for bulk and TF deposition data). Interaction between N_{inorg} and DON, tree species (pine, spruce) and DON, and latitude (north, south) and DON were tested. We used the Akaike information criterion (AIC) and residual deviance in comparing the models (the lower their values, the better the model predicted the BNF rate). The models were constructed in the R statistical environment (mgcv library) (R core team, 2017).

4. Results

4.1. Comparison of BNF rates between host species

All cryptogam species collected from two pine plots in the north showed a signal of BNF, i.e. a rate above the detection limit of 0.01 mg N kg^{-1} d^{-1} (Table 2). The highest BNF rates were measured in the feather mosses *P. schreberi* and *H. splendens*, which had similar mean rates (Table C.1). Also, their maximum rates of replicate samples were similar (8 mg N kg^{-1} d^{-1}) but found in different plots (Table A.1). Of the two liverwort species, *B. lycopodioides* had relatively high BNF rates in outdoor incubation, but *Ptilidium ciliare* showed activity only in a growth chamber (Table A.2). *Dicranum* spp. had a very low BNF rate, and *Cladina* spp. showed a signal just above the detection limit. Because between-sample variation within species was high, statistically significant differences were found only between the feather mosses and *Dicranum* spp. ($p < 0.05$) (Table C.1).

Latitude significantly explained the BNF rates of coexisting *H. splendens* and *P. schreberi*; i.e., the rates were higher in the north than in the south ($p < 0.01$), whereas the host bryophyte species or the dominant tree species had no effect (Table C.2). *H. splendens* was the only species expressing BNF activity in southern Finland, and thus showed the widest latitudinal range of BNF signal (Table 2).

4.2. BNF rates in relation to different forms of N deposition

The total amount of N increased from 0.8 to 4.4 kg ha^{-1} $year^{-1}$ in the bulk, and from 0.6 to 3.8 kg ha^{-1} $year^{-1}$ in the TF deposition along the north–south gradient (Fig. 2, Table 2). The BNF rates in *H. splendens*, *P. schreberi*, and *Dicranum* spp. decreased with increasing total N deposition in both bulk and TF deposition towards the south (Fig. 3). For *H. splendens*, the BNF activity was suppressed when the total N in bulk and TF deposition reached 3–4 kg N ha^{-1} $year^{-1}$. For the two other species, this level was even lower (2–3 kg N ha^{-1} $year^{-1}$), and these species had no signal in southern Finland (Table 2).

The absolute and relative amounts of different forms of N varied between bulk and TF deposition (Fig. 2a–b). In bulk, the proportion of N_{inorg} was 70–85% of total N, whereas in TF, it was around 60% in the north and only 40% in the southernmost plots. Both in bulk and TF, the amount of NO_3-N was higher than that of NH_4-N. Generally, the amount of DON increased clearly from north to south in TF, and it was higher in spruce stands than in pine stands. The BNF rates related to the total N as well as to different N components followed the shape of a negative exponent function for all three bryophyte species (Figs. 3, A.1, and A.2; Table C.5: models 1–7 for bulk and TF).

In one-predictor models N_{tot} (including DON) deposition did not explain better the decrease of the BNF rate than N_{inorg} deposition except in the TF model for *H. splendens* (curves and AIC values in Fig. 3). N_{inorg} was a significant predictor ($p < 0.01$) for the BNF rate in both bulk and TF deposition models for all three bryophyte species (Table 3: model 1). Adding DON as the second predictor (model 2) improved the model only in the case of TF for *H. splendens*, seen as lower AIC and deviance values (Fig. 3, Table 3). In this model, both N_{inorg} and DON were significant predictors ($p < 0.01$), indicating that both of them negatively affected the BNF rate in *H. splendens*. The interaction term between DON and tree species was significant ($p < 0.01$) when DON was not included as a main predictor (Table 3: model TF3 for *H. splendens*), showing that the negative effect of DON was larger in spruce stands than in pine stands.

4.3. Effect of temperature and moisture

The BNF rates in *H. splendens* and *P. schreberi* were significantly higher in warmer conditions in a growth chamber compared to the lower outdoor temperature ($p < 0.05$). *Dicranum* spp. showed a corresponding response to elevated temperature when comparing outdoor and indoor (laboratory room-temperature) incubations (Fig. 4a–c, Table C.3). A positive trend in the BNF rate with increased temperature was found in all plots studied.

The BNF rate of *P. schreberi* increased with increasing moisture ($p < 0.001$, Table C.4). The rate increased steeply from the air-dried samples to the field-moisture ones, and from there to samples that had received 1 ml of water. However, adding 2 ml or 5 ml of water did not increase the BNF rate any further than 1 ml of water (Fig. 4d).

5. Discussion

5.1. The effect of host species

All cryptogam species tested showed a capacity to host diazotrophic bacteria in the northern stands. However, there was high variation in

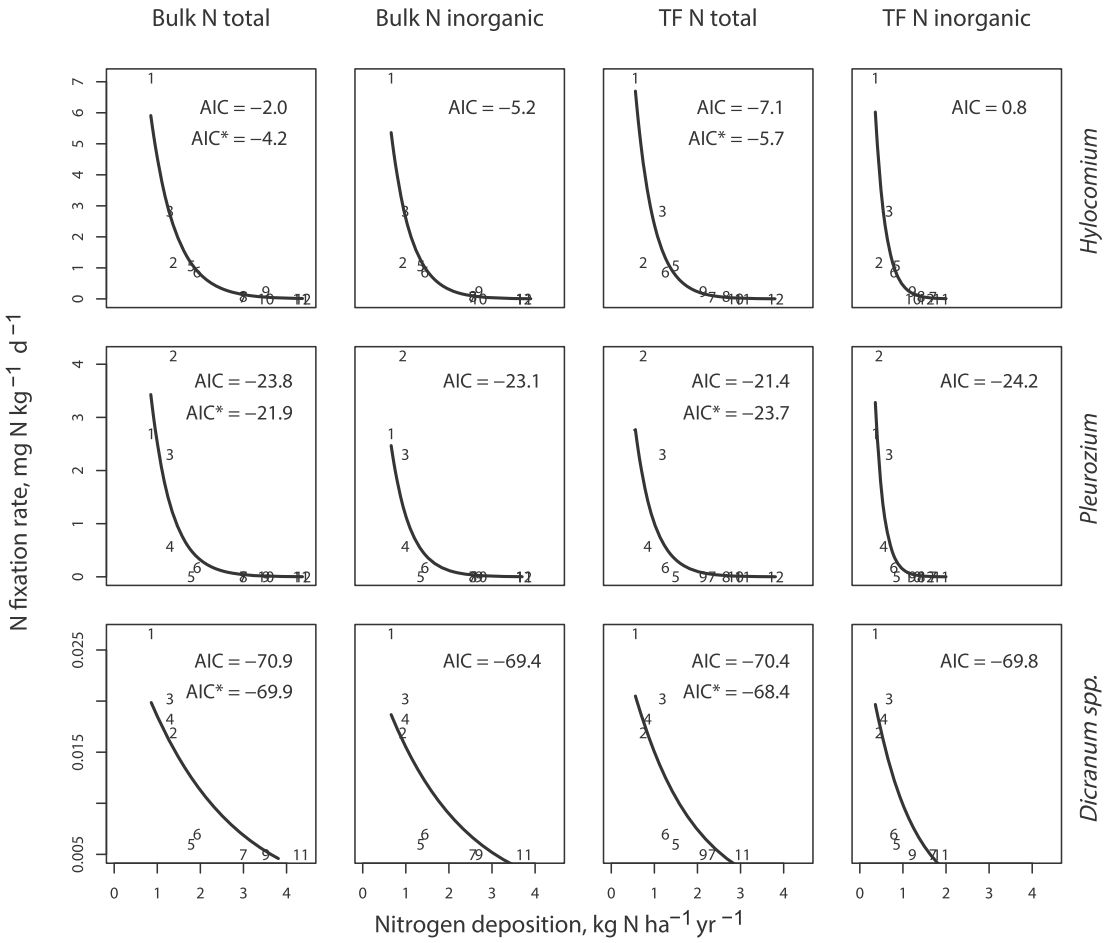


Fig. 3. Response curves for the average BNF rate (mg N kg moss^{-1} d^{-1}) of diazotrophs associated with bryophyte species (*Hylocomium splendens*, *Pleurozium schreberi* and *Dicranum* spp.) versus the total N (NH_4-N + NO_3-N + DON) and the inorganic N (NH_4-N, +NO_3-N) drawn separately for bulk and throughfall (TF) deposition (kg N ha^{-1} year^{-1}) along the climatic gradient in Finland. Sample plots (Fig. 1) are marked with numbers. Note the varying scales of the *y*-axes. The lower the Akaike's information criterion (AIC) value, the better the model predicts the BNF rate. AIC* values in the total N panels are for models with both N_{inorg} and DON as explanatory variables (Table 3). Species-specific curves for NH_4-N, NO_3-N and DON drawn separately for bulk and TF deposition are given in Figs. A.1 and A.2.

BNF rate between host species from different taxonomic and morphological groups. Such high variation was also reported by Gavazov et al. (2010) from material in northern Sweden. The host species were ordered according to BNF rate from high to low: *H. splendens* and *P. schreberi* > *B. lycopodioides* > *Dicranum* spp. > *Cladina* spp. This order may reflect the area of moisture-retaining surfaces on the species shoots or thallus (e.g. leaf–stem connections) offering suitable niches for epiphytic bacteria. As noted by Ininbergs et al. (2011) the composition of diazotrophic communities is highly host-specific, and this could also explain between-species differences in BNF rate.

In our study, the lichen species *Cladina stellaris* showed a weak signal of the occurrence of diazotrophs. Only a few studies have reported BNF activity in *Cladina* lichens, e.g. in *C. arbuscula* in Alpine conditions (Grube et al., 2009), and in *C. portentosa* in peatland conditions (van den Elzen et al., 2018). Similarly, *Dicranum* bryophyte species were not very significant hosts for diazotrophic bacteria: in northern species (e.g. *D. fuscescens*, *D. drummondii* and *D. bergeri*), BNF rates were low, and in southern *D. polysetum*, no BNF signal was found. Bay et al. (2013) observed that *D. polysetum* shoots collected from northern Sweden were not colonized by cyanobacteria, but interestingly, the shoots induced formation of hormogonia (cells with filaments enabling movement) in a *Nostoc* sp. culture more efficiently than feather mosses. This may indicate that *D. polysetum* has a capacity to tempt diazotrophs when it is N-limited. The basal parts of *D. scoparium* shoots showed a higher BNF rate than the upper parts (vice versa compared to feather mosses) in our test material. It is possible that epiphytic diazotrophs were colonized from the soil or that conditions for bacteria growth are more favorable (moister) near the soil layer. In contrast to the results from Gavazov et al. (2010), we also found BNF signals in two leafy liverwort species (*Barbilophozia lycopodioides* and *Ptilidium ciliare*). The BNF rate associated with *B. lycopodioides* was relatively high – almost a third of that measured on *P. schreberi* – while *P. ciliare* showed a signal only after acclimatizing to the high temperature in a growth chamber.

As part of our data, BNF activity in northern feather mosses has previously been shown to be cyanobacterial, representing mostly *Nostoc* and *Nodularia* genera (Leppänen et al., 2013). We had no information of the taxon of diazotrophs growing on *Dicranum* spp., liverwort species, and reindeer lichens. Other than associative cyanobacteria, free-living cyanobacteria or other N_2-fixing prokaryotes originating from soil (Rousk et al., 2013b), tree canopies (Lindo and Whiteley, 2011), or dead wood (Mäkipää et al., 2018) may also occur as casual epiphytes on cryptogam species.

5.2. The effect of nitrogen deposition

The BNF rates of diazotrophs associated with *H. splendens*, *P. schreberi* and *Dicranum* spp. decreased drastically with atmospheric N deposition, supporting the first hypothesis. Our results showed that diazotrophs of boreal bryophytes responded sensitively to the N availability they have been chronically exposed to in mature coniferous

Table 3
Comparison of the generalized linear models (GLM) explaining N_2 fixation rate associated with three bryophyte species (outdoor incubation) using either N_{inorg} deposition (NH_4-N + NO_3-N) or N_{inorg} and DON deposition as predictors (Table C.5: models 1 and 2 for bulk and TF). AIC coefficient, residual deviance and p values for N_{inorg} and DON (or DON:Tree interaction) are given. For *H. splendens*, interaction between DON and the dominant tree species of the plot (pine or spruce) was significant (model TF3), but N_{inorg}:DON and N_{inorg}:latitude interactions were insignificant. The models with the lowest AIC values gave the best fit to the data (marked in bold in feather mosses). n = number of plots per bryophyte species.

Species	Model	Precipitation	N form predictor	AIC	Deviance	p for N_{inorg}	p for DON
H. splendens n = 11	**B1**	**Bulk**	**N inorg**	**−5.2**	**7.1**	**0.001**	
	B2		N inorg + DON	−4.2	6.5	0.001	ns
	TF1	TF	N inorg	0.8	11.4	0.001	
	TF2		**N inorg + DON**	**−5.7**	**5.7**	**0.001**	**0.003**
	TF3		N inorg + DON:Tree	−2.8	7.3	0.001	0.01
P. schreberi n = 12	**B1**	**Bulk**	**N inorg**	**−23.1**	**19.8**	**0.001**	
	B2		N inorg + DON	−21.9	18.7	0.003	ns
	TF1	**TF**	**N inorg**	**−24.1**	**18.4**	**0.001**	
	TF2		N inorg + DON	−23.7	16.5	0.002	ns
Dicranum spp. n = 9	B1	Bulk	N inorg	−69.4	1.2	0.004	
	B2		N inorg + DON	−69.9	0.9	0.09	ns
	TF1	TF	N inorg	−69.8	1.2	0.003	
	TF2		N inorg + DON	−68.4	1.1	ns	ns

Models (Table C.5): (B1) BNF = glm (N_{inorg} in bulk, family = Gamma(link = "log")); (B2) BNF = glm (N_{inorg} in bulk + DON in bulk, family = Gamma(link = "log")); (TF1) BNF = glm (N_{inorg} in TF, family = Gamma(link = "log")); (TF2) BNF = glm (N_{inorg} in TF + DON in TF, family = Gamma(link = "log")); (TF3) BNF = glm (N_{inorg} in TF + DON in TF:Tree species, family = Gamma(link = "log")).

forests. Further, we concluded that the prevailing N deposition in their original growing conditions was the main factor that regulated the activity of diazotrophs on host species, which in turn explained the measured N_2 fixation. The effect of N deposition in field conditions existed, despite the fact that the samples were stored for 1–2 weeks in dark and cool conditions before the incubation experiment. It has been suggested that, apart from N, other elements e.g. heavy metals (Lorenz et al., 1992) may also have suppressing effects on BNF in diazotrophic bacteria. Deposition of heavy metals in background areas is currently very low in Finland (Poikolainen et al., 2004), and it is unlikely they have any effect on BNF in bryophytes.

The amount of total N was higher in bulk (consisting of much dry deposition) than in TF deposition, and the quantity and quality of forms of N changed greatly as TF filtrates through the canopy. The dominant role of DON in TF deposition in our study stands was in line with that measured in the N balance study of a Scots pine stand (SMEAR II station) near plot no. 8 in southern Finland (Korhonen et al., 2013). It is probable that the bryophyte layer is exposed mainly to bulk deposition when growing in stand openings, and to TF deposition under canopy. Because tree density was lower in the northern stands than the southern stands (shown by the stand basal area and canopy cover, Table 1), the canopy effect on the N deposition reaching the forest floor is less in the north than in the south.

P. schreberi and *Dicranum* spp. had no BNF activity in the southern plots, in contrast to *H. splendens*, which showed activity in all southern pine plots and in one spruce plot. The amount of N in TF deposition was lower in the southern pine than spruce stands. *H. splendens* had lower tissue N concentrations (mean 1.2%) compared to the other bryophyte species (1.4%) in pine stands (unpublished results). It is possible that a lower tissue N% indicated lower availability of N to epiphytic bacteria and enabled their BNF activity on *H. splendens* in pine plots. Its BNF signal was turned off at an N level of 3.3 kg ha^{-1} $year^{-1}$ in TF, and at 4.0 kg ha^{-1} $year^{-1}$ in bulk (calculated as the mean of the two plots

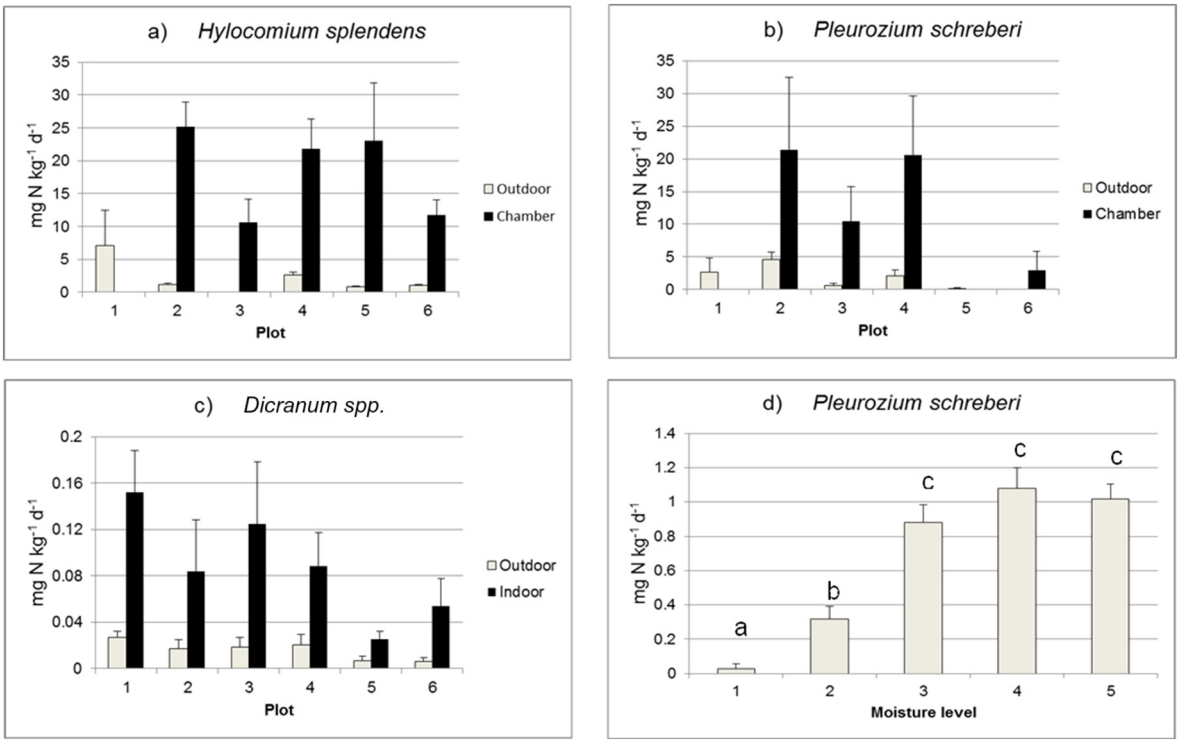


Fig. 4. The effect of temperature on the BNF rate (mg N kg^{-1} d^{-1}) in three bryophyte species collected from six northern plots (a–c), and the effect of moisture level on the BNF rate of *P. schreberi* from a northern spruce plot, no. 4 (d). Mean temperature was 9 °C (a, b) or 6 °C (c) outdoors ("low temperature"), 18–23 °C in the growth chamber, and 20 °C indoors ("high temperature"). Pairwise tests of BNF rates between two temperature conditions were statistically significant in all species and plots (a–c). The different letter above the bars indicates statically significant difference ($p < 0.05$) between means (d). Note the varying scales of the *y*-axes.

without signal) corresponding to 1.48% N in tissues (unpublished results). Generalizing the results over all three bryophyte species, we state the suppressing N level in bulk deposition for BNF activity of bryophyte-diazotroph associations is 3–4 kg ha^{-1} $year^{-1}$ in the boreal zone. This approximation is near the value of 4.5 kg N ha^{-1} $year^{-1}$ found to eliminate BNF in *P. schreberi* in a northern boreal forest (Zackrisson et al., 2004). Also Gundale et al. (2013) showed that an addition of 3 kg N ha^{-1} $year^{-1}$ significantly decreased BNF in *P. schreberi* in a field experiment. According to the regression model given by DeLuca et al. (2008), the BNF rate dropped by half from the maximum at an N amount of 6 kg ha^{-1} $year^{-1}$ (measured in TF *in situ* in the bryophyte layer). The suppressing N level for BNF activity in bryophytes found in this study was near the proposed critical N load of 2–3 kg ha^{-1} $year^{-1}$ for biodiversity of acidophytic lichens growing on tree trunks in boreal forests (Manninen, 2018).

According to the response models related to bulk deposition, N_{inorg} was a better predictor of BNF rate than N_{inorg} and DON together for all three bryophyte species (Fig. 3), which was against our second hypothesis. The result is reasonable, as most N in bulk deposition was in the form of NH_4-N and NO_3-N, and the amount of DON was low. Also, the TF models for *P. schreberi* and *Dicranum* spp. indicated that N_{inorg} was an adequate predictor of BNF rate. However, in the case of *H. splendens*, adding DON along with N_{inorg} improved the fit of the model, supporting the second hypothesis (Fig. 3). In the southern spruce stands, DON represented about half of the total N in TF. We conclude that DON had a negative effect on BNF, especially in spruce stands, as shown by the significant interaction between DON and tree species (Table 3).

Bryophytes assimilate selectively different forms of N from precipitation (Forsum et al., 2006; Nordin et al., 2006). In an N-uptake experiment, *H. splendens* preferred ^{15}N-labeled NH_4, and took up more glycine (important amino acid in DON) than NO_3-N from the spraying solutions representing N concentrations typical in precipitation in northern Sweden (Forsum et al., 2006). Rousk et al. (2013b) found that *P. schreberi* took up both NH_4-N and N-alanine from soil cores injected experimentally with various forms of N labeled with ^{15}N. Accordingly, our results may indicate that *H. splendens* adsorbed both inorganic and organic N from TF, because both forms of N were significant predictors of BNF rate in southern spruce stands. However, the negative effect of DON on BNF rates in bryophytes waits to be tested experimentally. Analyzing the composition of DON, instead of information about total DON only, would also increase our understanding on the inhibition phenomenon.

5.3. The effect of moisture and temperature

The BNF rates associated with bryophytes increased with increasing temperature in all three species, and with increasing moisture in our test species, *P. schreberi*, supporting the third hypothesis. These results are in accordance with earlier studies on the effects of temperature (e.g. Smith and Russell, 1982; Gentili et al., 2005; Gundale et al., 2012a), moisture amount and variability (Gundale et al., 2009; Jackson et al., 2011), and drying–rewetting cycle (Rousk et al., 2014b) on the BNF activity of feather mosses. The BNF rate in *P. schreberi* reached the steady state at 2 ml water addition, suggesting that the shoots were then saturated. We performed the outdoor incubations in quite low temperatures in August–October (mean 9.5 °C) compared to the known optimum temperature (25 °C) for the function of the enzyme nitrogenase (Houlton et al., 2008). Thus, it is probable that in our study, the BNF rates of bryophyte associations represented the lower level of the potential BNF range. On the other hand, the growth chamber temperature (18–23 °C) strongly activated BNF rates compared to the samples incubated outdoors. It seems that the BNF rates of chamber-incubated samples were unrealistically high to be measured in forests. It is true that many conditions in the chamber differed from those in field, e.g. shoots in incubation bottles were not exposed to drying. In addition, we cannot exclude possible effects of different light levels in outdoor and chamber experiments. According to Gundale et al. (2012a), a moderate increase in light activates BNF, but high light is detrimental.

The northern ecosystems are under the influence of a changing global climate. The recent scenarios for future climate predict that the average temperature in Finland will increase by 1–4 °C in summer and as much as 2–7 °C in winter. Elevated temperature may shorten the frost period of the soil, and this thaw will increase the moisture level in the bryophyte layer. Although the amount of precipitation in winter will increase, severe drought periods in summer will also become more frequent (Ruosteenoja et al., 2016). The physiological status of poikilohydric bryophytes readily follows changes in environmental conditions. It is probable that BNF rates in boreal bryophytes will increase in low-deposition areas if climate warming is linked to moister conditions. Also, biomass production of bryophytes may benefit from increased temperature if dryness is not a limiting factor (Hedwall et al., 2015). However, even a small increase in anthropogenic N deposition may counteract the effects of elevated temperature and moisture and suppress BNF activity, as shown in the data from southern Finland.

6. Conclusions

Our results demonstrated that biological N_2 fixation (BNF) in boreal bryophytes is sensitive to relatively low amounts of N deposition (3–-4 kg ha^{-1} $year^{-1}$) in field conditions in coniferous forests. Both inorganic and organic N suppressed the BNF rates, but their intercorrelated effects were complex, because tree canopy controlled the composition of nitrogen deposition. In northern ecosystems, BNF activity indicated bryophyte growth is N-limited, while lack of BNF signal in southern forests indicated N deposition is enough to satisfy the N demand of bryophytes. The BNF rate was also sensitive to changes in temperature and moisture level. We conclude that bryophyte BNF signals can be used as an indicator of anthropogenic N deposition load and climate change. As BNF rate is a measureable parameter, we suggest it should be incorporated into the critical N load calculations for the most sensitive organisms (bryophytes and lichens) in boreal forests.

Acknowledgements

We wish to thank the staff of the Natural Resources Institute Finland (Luke) for helping in the field work. We thank Anneli Rautiainen and Sanna Leppänen for their excellent work in the laboratory. Aleksi Lehtonen, Mikko Peltoniemi and Juha Heikkinen provided statistical advice in the data-handling stage. Sirkku Manninen kindly commented on the manuscript, and Nijole Kalinauskaite identified the *Dicranum* species and prepared the bryophyte drawing in the graphical abstract. A part of the data utilized in the study was collected with co-funding provided within the frameworks of the EU/Forest Focus (EC) No 2152/2003 and EU/Life+ FutMon programmes.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.10.364.

References

Ackermann, K., Zackrisson, O., Rousk, J., Jones, D.L., DeLuca, T.H., 2012. N2 fixation in feather mosses is a sensitive indicator of N deposition in boreal forests. Ecosystems 15, 986–998. https://doi.org/10.1007/s10021-012-9562-y.

Bay, G., Nahar, N., Oubre, M., Whitehouse, M.J., Wardle, D.A., Zackrisson, O., Nilsson, M., Rasmussen, U., 2013. Boreal feather mosses secrete chemical signals to gain nitrogen. New Phytol. 200, 54–60. https://doi.org/10.1111/nph.12403.

Cornell, S.E., Jickells, T.D., Cape, J.N., Rowland, A.P., Duce, R.A., 2003. Organic nitrogen deposition on land and coastal environments: a review of methods and data. Atmos. Environ. 37, 2173–2191. https://doi.org/10.1016/S1352-2310(03)00133-X.

DeLuca, T.H., Zackrisson, O., Nilsson, M.-C., Sellstedt, A., 2002. Quantifying nitrogen–fixation in feather bryophyte carpets of boreal forests. Nature 419, 917–920. https://doi.org/10.1038/nature01051.

DeLuca, T.H., Zackrisson, O., Gundale, M.J., Nilsson, M.-C., 2008. Ecosystem feedbacks and nitrogen fixation in boreal forests. Science 320 (5880), 1181. https://doi.org/10.1126/science.1154836.

Ferm, M., Hultberg, H., 1999. Dry deposition and internal circulation of nitrogen, sulphur and base cations to a coniferous forest. Atmos. Environ. 33, 4421–4430. https://doi.org/10.1016/S1352-2310(99)00211-3.

Forsum, Å., Dahlman, L., Näsholm, T., Nordin, A., 2006. Nitrogen utilization by *Hylocomium splendens* in a boreal forest fertilization experiment. Funct. Ecol. 20, 421–426. https://doi.org/10.1111/j.1365-2435.2006.01127.x.

Gavazov, K.S., Soudzilovskaia, N.A., van Logtestijn, R.S.P., Braster, M., Cornelissen, J.H.C., 2010. Isotopic analysis of cyanobacterial nitrogen fixation associated with subarctic lichen and bryophyte species. Plant Soil 333, 507–517. https://doi.org/10.1007/s11104-010-0374-6.

Gentili, F., Nilsson, M.-C., Zackrison, O., DeLuca, T.H., Sellstedt, A., 2005. Physiological and molecular diversity of feather moss associative N_2-fixing cyanobacteria. J. Exp. Bot. 56, 3121–3127. https://doi.org/10.1093/jxb/eri309.

Grube, M., Cardinale, M., de Castro Jr., J.V., Müller, H., Berg, G., 2009. Species-specific structural and functional diversity of bacterial communities in lichen symbioses. ISME J. 3 (9), 1105–1115. https://doi.org/10.1038/ismej.2009.63.

Gundale, M.J., Gustafsson, H., Nilsson, M.C., 2009. The sensitivity of nitrogen fixation by a feathermoss–cyanobacteria association to litter and moisture variability in young and old boreal forest. Can. J. For. Res. 39, 2542–2549. https://doi.org/10.1139/X09-160.

Gundale, M.J., DeLuca, T.H., Nordin, A., 2011. Bryophytes attenuate anthropogenic nitrogen inputs in boreal forests. Glob. Chang. Biol. 17, 2743–2753. https://doi.org/10.1111/j.1365-2486.2011.02407.x.

Gundale, M.J., Wardle, D.A., Nilsson, M.-C., 2012a. The effect of altered macroclimate on N-fixation by boreal feather mosses. Biol. Lett. 8, 805–808. https://doi.org/10.1098/rsbl.2012.0429.

Gundale, M.J., Nilsson, M., Bansal, S., Jäderlund, A., 2012b. The interactive effects of temperature and light on biological nitrogen fixation in boreal forests. New Phytol. 194, 453–463. https://doi.org/10.1111/j.1469-8137.2012.04071.x.

Gundale, M.J., Bach, L.H., Nordin, A., 2013. The impact of simulated chronic nitrogen deposition on the biomass and N_2-fixation activity of two boreal feather moss-cyanobacteria associations. Biol. Lett. 9, 20130797. https://doi.org/10.1098/rsbl.2013.0797.

Hedwall, P.O., Skoglund, J., Linder, S., 2015. Interactions with successional stage and nutrient status determines the life-form-specific effects of increased soil temperature on boreal forest floor vegetation. Ecol. Evol. 5, 948–960. https://doi.org/10.1002/ece3.1412.

Houlton, B.Z., Wang, Y.P., Vitousek, P.M., Field, C.P., 2008. A unifying framework for dinitrogen fixation in the terrestrial biosphere. Nature 454, 327–330. https://doi.org/10.1038/nature07028.

Ininbergs, K., Bay, G., Rasmussen, U., Wardle, D.A., Nilsson, M.-C., 2011. Composition and diversity of nifH genes of nitrogen-fixing cyanobacteria associated with boreal forest feather mosses. New Phytol. 192, 507–517. https://doi.org/10.1111/j.1469-8137.2011.03809.x.

IPCC, 2013. Climate Change 2013: The Physical Science Basis. Annex I: Atlas of Global and Regional Climate Projections. , pp. 1350–1353 https://www.ipcc.ch/pdf/assessment-report/ar5/wg1/WG1AR5_AnnexI_FINAL.pdf.

Jackson, B.G., Martin, P., Nilsson, M.C., Wardle, D.A., 2011. Response of feather moss associated N2 fixation and litter decomposition to variations in simulated rainfall intensity and frequency. Oikos 120, 570–581. https://doi.org/10.1111/j.1600-0706.2010.18641.x.

Korhonen, J.F.J., Pihlatie, M., Pumpanen, J., Aaltonen, H., Hari, P., Levula, J., Kieloaho, A.-J., Nikinmaa, E., Vesala, T., Ilvesniemi, H., 2013. Nitrogen balance of a boreal Scots pine forest. Biogeosciences 10, 1083–1095. https://doi.org/10.5194/bg-10-1083-2013.

Leppänen, S.M., Salemaa, M., Smolander, A., Mäkipää, R., Tiirola, M., 2013. Nitrogen fixation and methanotrophy in forest mosses along a N deposition gradient. Environ. Exp. Bot. 90, 62–69. https://doi.org/10.1016/j.envexpbot.2012.12.006.

Lindo, Z., Whiteley, J.A., 2011. Old trees contribute bio-available nitrogen through canopy bryophytes. Plant Soil 342, 141–148. https://doi.org/10.1007/s11104-010-0678-6.

Lindo, Z., Griffith, D.A., 2017. Elevated atmospheric CO2 and warming stimulates growth and nitrogen fixation in a common forest floor cyanobacterium under axenic conditions. Forests 8, 73. https://doi.org/10.3390/f8030073.

Lindo, Z., Nilsson, M.-C., Gundale, M.J., 2013. Bryophyte-cyanobacteria associations as regulators of the northern latitude carbon balance in response to global change. Glob. Chang. Biol. 19, 2022–2035. https://doi.org/10.1111/gcb.12175.

Lorenz, S.E., McGrath, S.P., Giller, K., 1992. Assessment of free-living nitrogen fixation activity as a biological indicator of heavy metal toxicity in soil. Soil Biol. Biochem. 24, 601–606. https://doi.org/10.1016/0038-0717(92)90086-D.

Mäkipää, R., Leppänen, S.M., Sanz Munoz, S., Smolander, A., Tiirola, M., Tuomivirta, T., Fritze, H., 2018. Methanotrophs are core members of the diazotroph community in decaying Norway spruce logs. Soil Biol. Biochem. 120, 230–232. https://doi.org/10.1016/j.soilbio.2018.02.012.

Manninen, S., 2018. Deriving nitrogen critical levels and loads based on the responses of acidophytic lichen communities on boreal urban *Pinus sylvestris* trunks. Sci. Total Environ. 613–614, 751–762. https://doi.org/10.1016/j.scitotenv.2017.09.150.

Merilä, P., Mustajärvi, K., Helmisaari, H.-S., et al., 2014. Above- and below-ground N stocks in coniferous boreal forests in Finland: implications for sustainability of more intensive biomass utilization. For. Ecol. Manag. 311, 17–28. https://doi.org/10.1016/j.foreco.2013.06.029.

Mustajärvi, K., Merilä, P., Derome, J., Lindroos, A.-J., Helmisaari, H.-S., Nöjd, P., Ukonmaanaho, L., 2008. Fluxes of dissolved organic and inorganic nitrogen in relation to stand characteristics and latitude in Scots pine and Norway spruce stands in Finland. Boreal Environ. Res. 13 (Suppl. B), 3–21.

Neff, J.C., Holland, E.A., Dentener, F.J., Mcdowell, W.H., Russell, K.M., 2002. The origin, composition and rates of organic nitrogen deposition: a missing piece of the nitrogen cycle? Biogeochemistry 57 (58), 99–136.

Nordin, A., Strengbom, J., Ericson, L., 2006. Responses to ammonium and nitrate additions by boreal plants and their natural enemies. Environ. Pollut. 141, 167–174. https://doi.org/10.1016/j.envpol.2005.08.017.

Poikolainen, J., Kubin, J., Piispanen, J., Karhu, J., 2004. Atmospheric heavy metal deposition in Finland during 1985–2000 using mosses as bioindicators. Sci. Total Environ. 318, 171–185. https://doi.org/10.1016/0038-0717(92)90086-D.

Proctor, M.C.F., Oliver, M.J., Wood, A.J., Alpert, P., Stark, L.R., Cleavitt, N.L., Mishler, B.D., 2007. Desiccation-tolerance in bryophytes: a review. Bryologist 110, 595–621. https://doi.org/10.1639/0007-2745(2007)110[595:DIBAR]2.0.CO;2.

R Development Core Team, 2017. https://www.R-project.org.

Rikkinen, J., 2017. Cyanobacteria in terrestrial symbiotic systems. In: Hallenbeck, P.C. (Ed.), Modern Topics in the Phototrophic Prokaryotes. Springer, pp. 243–294 https://doi.org/10.1007/978-3-319-46261-5_8.

Rousk, K., Michelsen, A., 2016. The sensitivity of moss-associated nitrogen fixation towards repeated nitrogen input. PLoS One https://doi.org/10.1371/journal.pone.0146655 (January 5).

Rousk, K., Jones, D.L., DeLuca, T.H., 2013a. Moss-cyanobacteria associations as biogenic sources of nitrogen in boreal forest ecosystems. Front. Microbiol. 4, 150. https://doi.org/10.3389/Ffmicb.2013.00150.

Rousk, K., Rousk, J., Jones, D.L., Zackrisson, O., DeLuca, T.H., 2013b. Feather moss nitrogen acquisition across natural fertility gradients in boreal forests. Soil Biol. Biochem. 61, 86–97. https://doi.org/10.1016/j.soilbio.2013.02.011.

Rousk, K., Jones, D.L., DeLuca, T.H., 2014a. Exposure to nitrogen does not eliminate N_2 fixation in the feather moss *Pleurozium schreberi* (Brid.) Mitt. Plant Soil 374, 513–521. https://doi.org/10.1007/s11104-013-1908-5.

Rousk, K., Jones, D.L., DeLuca, T.H., 2014b. The resilience of nitrogen fixation in feather moss (*Pleurozium schreberi*) – cyanobacteria associations after a drying and rewetting cycle. Plant Soil 377, 159–167. https://doi.org/10.1007/s11104-013-1984-6.

Ruoho-Airola, T., Syri, S., Nordlund, G., 1998. Acid deposition trends at the Finnish Integrated Monitoring catchments in relation to emission reductions. Boreal Environ. Res. 3, 204–219.

Ruosteenoja, K., Jylhä, K., Kämäräinen, M., 2016. Climate projections for Finland under the RCP forcing scenarios. Geophysica 51, 17–50.

Salemaa, M., Derome, J., Nöjd, P., 2008. Response of boreal forest vegetation to the fertility status of the organic layer along a climatic gradient. Boreal Environ. Res. 13 (Suppl. B), 48–66.

Smith, V.R., Russell, S., 1982. Acetylene reduction by bryophyte-cyanobacteria associations on a subantarctic island. Polar Biol. 1, 153–157. https://doi.org/10.1007/BF00287001.

STATISTIX 10.0. Analytical sofware. https://www.statistix.com.

Stuiver, B.M., Gundale, M.J., Wardle, D.A., Nilsson, M.-C., 2015. Nitrogen fixation rates associated with the feather mosses *Pleurozium schreberi* and *Hylocomium splendens* during forest stand development following clear-cutting. For. Ecol. Manag. 347, 130–139. https://doi.org/10.1016/j.foreco.2016.05.038.

van den Elzen, E., van den Berg, L.J., van der Weijden, B., Fritz, C., Sheppard, L.J., Lamers, L.P.M., 2018. Effects of airborne ammonium and nitrate pollution strongly differ in peat bogs, but symbiotic nitrogen fixation remains unaffected. Sci. Total Environ. 610, 732–740. https://doi.org/10.1016/j.scitotenv.2017.08.102.

Venäläinen, A., Tuomenvirta, H., Pirinen, P., Drebs, A., 2005. A basic climate data set 1961–2000–description and illustrations. Finnish Meteorological Institute Reports vol. 5, p. 27.

Vuorenmaa, J., Augustaitis, A., Beudert, B., Clarke, N., de Witt, H.A., Dirnböck, T., Frey, J., Forsius, M., Indriksone, I., Kleemola, S., Kobler, J., Krám, P., Lindroos, A.-J., Lundin, L., Ruoho-Airola, T., Ukonmaanaho, L., Vana, M., 2017. Long-term sulphate and inorganic nitrogen mass balance budgets in European ICP Integrated Monitoring catchments (1990–2012). Ecol. Indic. 76, 15–29. https://doi.org/10.1016/j.ecolind.2016.12.040.

Vuorenmaa, J., Augustaitis, A., Beudert, B., Bochenek, W., Clarke, N., de Wit, H.A., Dirnböck, T., Frey, J., Hakola, H., Kleemola, S., Kobler, J., Kram, P., Lindroos, A.-J., Lundin, L., Löfgren, S., Marchetto, A., Pecka, T., Schulte-Bisping, H., Skotak, K., Srybny, A., Szpikowski, J., Ukonmaanaho, L., Vana, M., Åkerholm, S., Forsius, M., 2018. Long-term changes (1990–2015) in the atmospheric deposition and runoff water chemistry of sulphate, inorganic nitrogen and acidity for forested catchments in Europe in relation to changes in emissions and hydrometeorological conditions. Sci. Total Environ. 625, 1129–1145. https://doi.org/10.1016/j.scitotenv.2017.12.245.

Waldner, P., Marchetto, A., Thimonier, A., Schmitt, M., et al., 2014. Detection of temporal trends in atmospheric deposition of inorganic nitrogen and sulphate to forests in Europe. Atmos. Environ. 95, 363–374. https://doi.org/10.1016/j.atmosenv.2014.06.054.

Zackrisson, O., DeLuca, T.H., Nilsson, M.-C., Sellstedt, A., Berglund, L.M., 2004. Nitrogen fixation increases with successional age in boreal forests. Ecology 85, 3327–3334. https://doi.org/10.1890/04-0461.